Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.101222

Study on meat color stability of Qinchuan cattle during post-slaughter storage

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Abstract

To study the stability of meat color in the post-slaughter storage process of Qinchuan cattle, three different bovine muscles, including longissimus dorsi (LD), knee round (KR) and psoas major (PM) muscles of Qinchuan cattle, were selected as research objects to determine the changes in meat color, myoglobin (Mb) relative content, pH, oxygen consumption rate (OCR), total iron, heme iron (HI) and other contents during storage. With the prolongation of the storage time, the relative content of oxymyoglobin (OxyMb) decreased, the relative content of metmyoglobin (MetMb) gradually increased, and muscle browning was due to the increase of a^* value and hue value; Among them, HI and non-heme iron (NHI) reduced the stability of meat color by accelerating lipid oxidation, which was consistent with the results of Mb content and meat color index determining meat color stability. Therefore, the total iron content in muscles can also be used as an indicator of meat color stability.

Keywords: different parts; myoglobin; iron; meat color stability.

Practical Application: At present, there are few reports of the effects of HI and NHI on the color stability of cattle during storage. Therefore, this paper selects the LD, PM and KR muscles of Qinchuan cattle as the research objects to observe the color changes, which provides theoretical support for further study of the differential mechanism of meat color stability in different parts of the muscles.

1 Introduction

Qinchuan cattle are mostly produced in Shaanxi, Gansu and Ningxia, and are one of the dominant characteristic livestock breeds in northwest China. Because of its delicate meat quality, rich meaty flavor, high lean meat rate, obvious marble pattern and high nutritional value, it is highly sought after by consumers. Chilled Qinchuan cattle are similar in quality and traits to fresh cattle, so it is favored.

Meat color is the most intuitive indicator of meat quality and is the main factor affecting consumers' desire to buy (Testa et al., 2021). Discolored meat is usually processed into minced meat or other products and sold at a discount, resulting in greater losses (Ramanathan et al., 2021). However, the discoloration of meat surface is inevitable, and the discoloration time of fresh meat is short (Singh et al., 2022; Turan & Şimşek, 2021). Therefore, it is becoming more and more important to evaluate the factors that affect the stability of meat color. Li et al. (2021) showed that the accumulation rate of MetMb in cattle decreased after high-pressure treatment during storage, and high-pressure treatment improved the stability of meat color during storage. Yang et al. (2021) found that calcium lactate treatment significantly reduced the meat color deterioration rate of cattle during cold storage, and significantly improved meat color stability. Yang et al. (2022) found that the effect of high oxygen packaging on a steak proteome during storage will directly determine the change of meat color stability. During storage, the MetMb reduction ability and OCR of high oxygen steak decreased rapidly with the extension of storage time, the relative MetMb content increased significantly, and the meat color stability of steak decreased continuously. At present, most studies are to improve the stability of meat color by reducing the activity of MetMb reductase. Some studies have shown that the color stability of meat depends not only on the species (Bechtold et al., 2019; Lima et al., 2021), but also on the muscle specificity (Nair et al., 2016; Salim et al., 2019; Wu et al., 2016). Therefore, different strategies are needed to improve the meat color stability of different varieties and muscles.

Some studies have shown that HI and NHI, as oxidation products of Mb and hemoglobin (Hb), participate in free radical chain reactions to form lipid and protein oxidation products, which can weaken the oxidative stability of the muscles (Li, 2022).

2 Materials and methods

2.1 Materials and reagents

Material: Qinchuan cattle are provided by Xukang Food Co., LTD., Jingchuan County, Pingliang City, Gansu Province. Six 2-year-old 400 kg bulls were slaughtered under the same feeding and management conditions. They were fasted for 18 hours and cut off water for 2 hours before slaughter. The LD, PM and KR muscles were taken within 30 min after slaughter.

Received 15 Sept., 2022
Accepted 07 Nov., 2022
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Reagents: Sodium dihydrogen phosphate, disodium hydrogen phosphate, hydrochloric acid, nitric acid and acetone (analytical purity) were all purchased from China Pharmaceutical Group Chemical Reagent Co., Ltd.

2.2 Sample processing

After quickly removing the surface fat, tendons and connective tissue, it was divided into about 100 g meat samples, and then packaged in self-sealing bags and tinfoil on the spot. After vacuum packaging, it was stored at 4 °C, and samples were taken at 1, 3, 5 and 7 d after slaughter.

2.3 Determination of flesh color

The L^*, a^* and b^* were measured with a chromometer (MA-T6, Nanjing, China). Before measurement, the whiteboard was used to correct the colorimeter, the light source was D65, and the measurement diameter was 8 mm. After the meat sample cutting surface was in contact with the oxygen in the air 30 min, parallel measurements were carried out on three different parts to be tested, and each sample was measured three times and the average value was taken. The formulas to calculate the Chroma (C*) and Hue angle (H) are as follows (Equations 1 and 2):

$$C^* = (a^{*2} + b^{*2})^{0.5} \tag{1}$$

$$H = \arctan\frac{b^*}{a^*} \tag{2}$$

2.4 Determination of three kinds of Mb contents

Slightly modified according to the methods of Bai et al. (2022) and Xia et al. (2020), 5 g minced meat was taken and added with 25 mL phosphate buffer (0.04 mol/L, pH 6.8). After homogenization (JXFSTPRP-CL, Refrigeration grinder, Shanghai, China) for 25 s, the homogenized solution was placed in a refrigerator at 4 °C for 1 h, centrifuged under 8000 r/min for 25 min (TGL-24M, High-speed refrigerated centrifuge, Changsha, China). The supernatant was filtered and the absorbance of the filtrate was determined at the wavelengths of 525, 545, 565 and 572 nm, respectively (UV-1200, spectrophotometer, Shanghai, China). The calculation formulas of three kinds of Mb contents are as follows (Equations 3, 4, 5 and 6):

$$C_{Mb} = -0.166_{A572} + 0.086_{A565} + 0.088_{A545} + 0.099_{A525} \tag{3}$$

$$P_1 = \left(0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015\right)\times 100 \tag{4}$$

$$P_2 = \left(0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361\right)\times 100 \tag{5}$$

$$P_3 = \left(-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098\right)\times 100 \tag{6}$$

In formulas, P_1, P_2 and P_3 are the mass fraction of deoxymyoglobin (DeoxyMb), OxyMb and MetMb, respectively, and R_1, R_2 and R_3 are absorbance ratios A_{572}/A_{525}, A_{565}/A_{525} and A_{545}/A_{525}, respectively.

2.5 Determination of pH value

Refer to the method of Oliveira et al. (2022). The calibrated pH meter probe (SJ-3F, Shanghai, China) was inserted into the meat, and three different positions were selected for determination, the results were averaged.

2.6 OCR treatment

Refer to the method of Gao et al. (2013) and modify it slightly. Expose the meat sample (3 × 3 × 2 cm) to oxygen for 30 min, vacuum immediately, and measure the surface reflectivity (400~700 nm) after packaging to calculate the initial OxyMb%. The sample was heated for 20 min in a water bath at 25 °C, and the surface reflectivity was measured again to calculate the final OxyMb%. Do it three times in parallel, and the results are averaged. The calculation formula of OCR is as follows (Equation 7):

$$OCR\ /\ \% = \frac{\text{initial OxyMb\%-final OxyMb\%}}{\text{initial OxyMb\%}}\times 100 \tag{7}$$

2.7 Total iron, HI and NHI determinations

The total iron content in meat was determined according to Schricker et al. (1982). After the fat was removed, the meat samples were crushed, freeze-dried (FD-1A-50, Freeze dryer, Lanzhou, China) for 9 h and a ground to a powder using a freeze grinder. The content of HI was determined by Farzin et al. (2016). Take 0.5 g powder sample into 10 mL centrifuge tube, add 5 mL acid acetone solution, mix, shake in water bath and centrifuge for 3 min at 7000 r/min, collect acid acetone layer in triangle flask, add 5 mL of acid acetone to the remaining solids of the centrifuge tube, centrifuge separation after repeated oscillation extraction, combine acid acetone layer in triangle flask, put acetone in 60 °C water bath to remove acetone until the solution volume is 4~6 mL, cool at room temperature. Digestion assays were performed by the method for determining total iron content. The content of NHI is calculated by the following formula (Equation 8):

$$\text{NHI content} = \text{total iron content} - \text{HI content} \tag{8}$$

2.8 Data processing

SPSS 26.0 software was used to analyze the variance and the significance of the experimental data, and the difference was significant when $P < 0.05$. Origin 2022 software was used for drawing experimental results.

3 Result and discussion

3.1 Changes of cattle color in different parts during storage

On the first day, the L^* value of the LD muscle was significantly higher than that of the other two parts ($P < 0.05$) (Figure 1A), indicating that LD muscle had better brightness at the beginning of storage. The L^* values of PM and KR muscles, increased sharply in 1~3 d, and decreased slowly in 3~5 d, but the change of L^* value of PM muscle was not significant. The L^* value of LD muscle increased slowly in 1~5 d. From 5 d

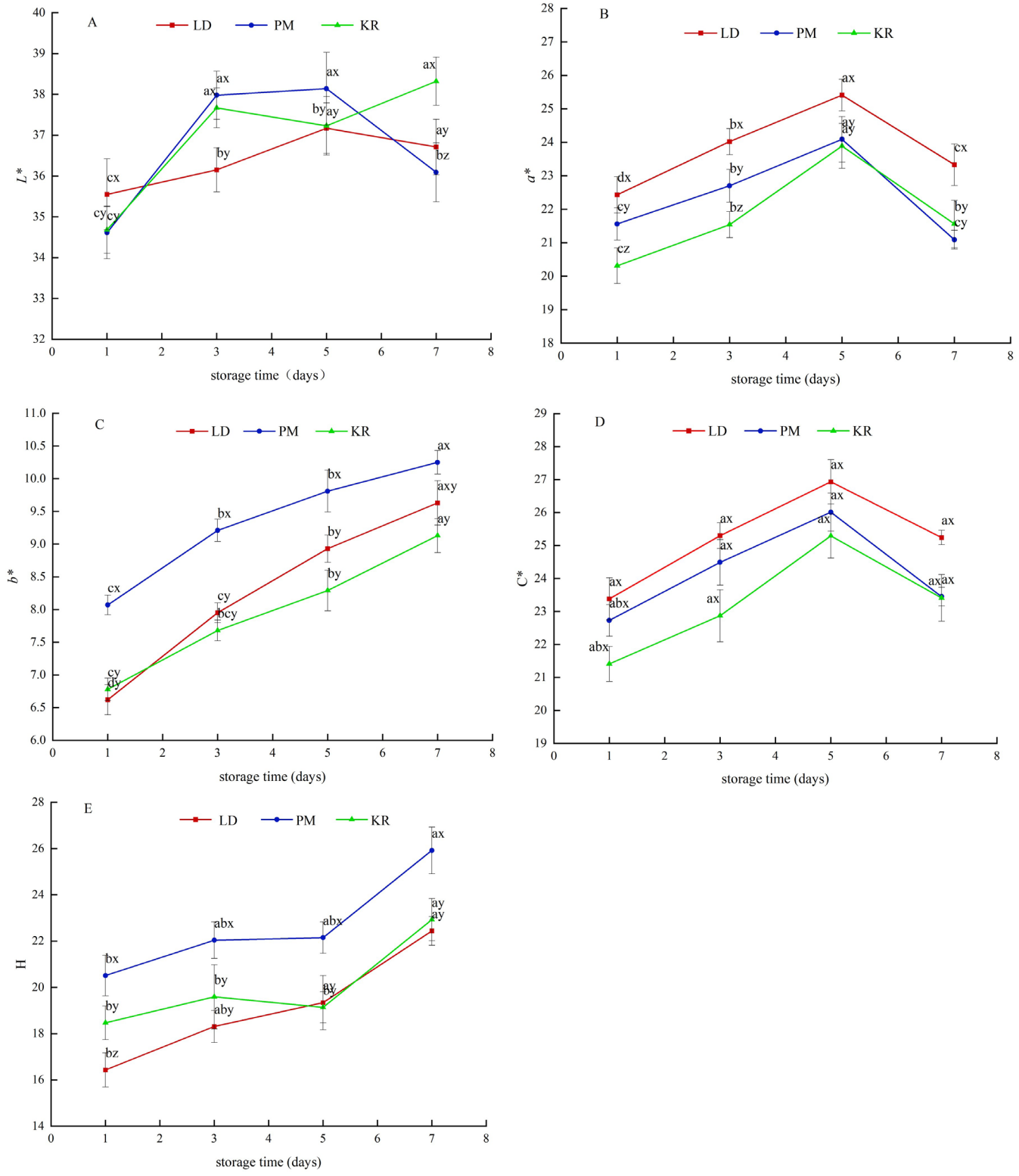

Figure 1. Variation of (A) L^* value, (B) a^* value, (C) b^* value, (D) C*, (E) H in different parts of cattle during storage. Note: different letters (a~c) indicate significant difference of the same muscle of cattle in different storage times ($P < 0.05$), while different letters (x~z) indicate significant difference of muscles in different parts at the same time ($P < 0.05$). The same below.

to 7 d, the L^* values of LD and PM muscles decreased, while that of KR muscle increased. The L^* values of the three parts were different at 7 d ($P < 0.05$), among which the L^* values of KR muscles were the highest and those of PM muscles were the

lowest. It shows that the stability of the L^* value of PM muscle is the worst in 1~7 d. On the 1st day, the a^* values of the three parts were significantly different ($P < 0.05$) (Figure 1B). From 1 d to 5 d, the a^* values of the muscles of the three parts showed an increasing trend, indicating that a large number of OxyMb was formed, which made the cattle show bright red. Among them, the growth range of the LD muscle was similar to that of the PM muscle, and the a^* value of KR muscle increased sharply in 3~5 d. The a^* values of the muscles of the three parts decreased from 5 d to 7 d, and the a^* value of the LD muscle was the highest ($P < 0.05$). There was no significant difference between KR and PM muscles, indicating that the LD muscle had good meat color stability. On the 1st day, the b^* value of PM muscle was different from those of LD and KR muscles ($P < 0.05$), but there was no significant difference between LD and KR muscles (Figure 1C). In 1~7 d, the b^* values of the three parts showed an upward trend, this is because with the extension of storage time, MetMb in muscle gradually accumulated, showing an increase in b^* value. At 7 d, the b^* values of muscles in three parts were significantly different from those at 1 d ($P < 0.05$), in which the b^* value of PM muscle was the highest and KR muscle was the lowest. It shows that the stability of b^* value of PM muscle is the worst. There was no significant difference in chroma among the three muscles at 1 d (Figure 1D). The change trend of chroma of the three different parts was the same in 1~7 d, rising in 1~5 d and decreasing in 5~7 d, indicating that the discoloration of muscle began to occur from the 5th day, and the meat color stability of cattle decreased continuously in 5~7 d. Among them, the LD muscle has better chroma and a^* than the other two muscles, indicating that the LD muscle has more saturated red and better flesh color stability. The change trend of hue angle of muscle in three parts is the same (Figure 1E). There were significant differences in hue angles among the three parts ($P < 0.05$). The hue angle of PM muscle was always higher than that of the other two muscles ($P < 0.05$). At 5~7 d, there was no significant difference in hue angle between KR and LD muscles. Overall, the flesh color stability of these three parts from high to low is: LD, KR, PM muscle. Because the muscles of different parts have different physiological functions in vivo, there are great differences in the proportion of fiber types and the final muscle fiber types (Fang et al., 2022), thus showing different flesh color stability after slaughtering.

3.2 Changes in relative content of cattle Mb in different parts during storage

Mb is a major factor affecting muscle color (Cross et al., 2017; Liu et al., 2021). The color of meat is affected by the concentration and physicochemical state of Mb. The relative content of OxyMb in three different parts of muscle showed the downward trend from 1 d to 7 d (Figure 2A). On the 1st day, there was no difference in the relative content of OxyMb in three parts of the muscle, but on the 7th day, the relative content of OxyMb in the LD muscle was significantly different from that in the other two parts ($P < 0.05$). The relative content of OxyMb in three parts of the 7th day was significantly different from that on the 1st day ($P < 0.05$). The relative content of DeoxyMb in three different parts of muscles showed the same trend from 1 d to 7 d (Figure 2B). There was no difference in the relative content of DeoxyMb in the three parts of muscle from 1 d to 7 d, but the relative content of OxyMb in the three parts of the 7th day was significantly different from that on the 1st day ($P < 0.05$). The relative content of MetMb in three different parts of muscle showed an upward trend from 1 d to 7 d (Figure 2C). On the 1st day, the relative content of MetMb in the LD muscle was significantly different from that in the other two parts. On the 7th day, the relative content of MetMb in the PM muscle was significantly different from that in the other two parts. The relative content of MetMb in the PM muscle was the highest and that of the LD muscle was the lowest. OxyMb is related to L^*, a^*, b^*, hue angle and chroma (Calnan et al., 2016). When DeoxyMb in muscle combines with oxygen to form OxyMb, the color of meat will change from fuchsia to bright cherry red, and the L^* value will increase due to Mb oxygenation (Hasan et al., 2021). With the extension of muscle storage time, OxyMb was oxidized from Fe^{2+} to Fe^{3+}, forming MetMb, and the color of meat gradually turned brown. Therefore, with the decrease of the relative content of OxyMb and the gradual increase of MetMb in muscle, the a^* value showed a downward trend (Figure 1B) (Ramanathan et al., 2020; Santos et al., 2021). Meantime, the browning of the meat surface increases. Over time, the relative content of MetMb (Figure 2C) and hue angle (Figure 1E) of all muscles increased, which could be seen the increase of browning during storage. The relative content of OxyMb in the LD muscle was higher than that in the other two muscles on the 7th day, the relative content of DeoxyMb and MetMb in the LD muscle was lower than that in the other two muscles, indicating that the meat color stability of the LD muscle was better, while that of the PM muscle was the worst. Because the LD muscle has the lowest hue angle and relative content of MetMb, the meat color stability of LD muscle is the best among the three kinds of muscles.

3.3 Changes in pH of cattle in different parts during storage

Gagaoua et al. (2017) showed that pH value had a significant effect on the color variation of all muscles. Since the decrease of pH value of muscle was accompanied by changes in muscle fiber structure and filament arrangement, it might change the light scattering on the muscle surface, affecting the chromatic aberration measured by the instrument. As can be seen from Figure 3, the pH values of the three parts first decreased and then increased in 1~7 d. The pH value of KR muscle increased after 3 d, and the pH of LD and PM muscles increased after 5 d. On the 7th day, the pH value of LD and PM muscles was significantly higher than that of KR muscle ($P < 0.05$). Studies have shown that high pH value leads to lower L^* value and darker color of the muscles, and vice versa (Warner et al., 2014; Zhang et al., 2018), which is consistent with the results of this experiment, where the KR muscle has the highest L^* value (Figure 1A) and the lowest pH value (Figure 3). A study has shown that high pH value will minimize the oxidation of Mb and keep muscle bright red for a long time, while lower pH value will accelerate the oxidation of Mb and lead to meat color browning (Hasan et al., 2021). Because the LD muscle has higher pH value and lower relative content of MetMb, the meat color stability of LD muscle is the best. Similarly, the meat color stability of KR muscle is the second, and PM muscle is the worst.

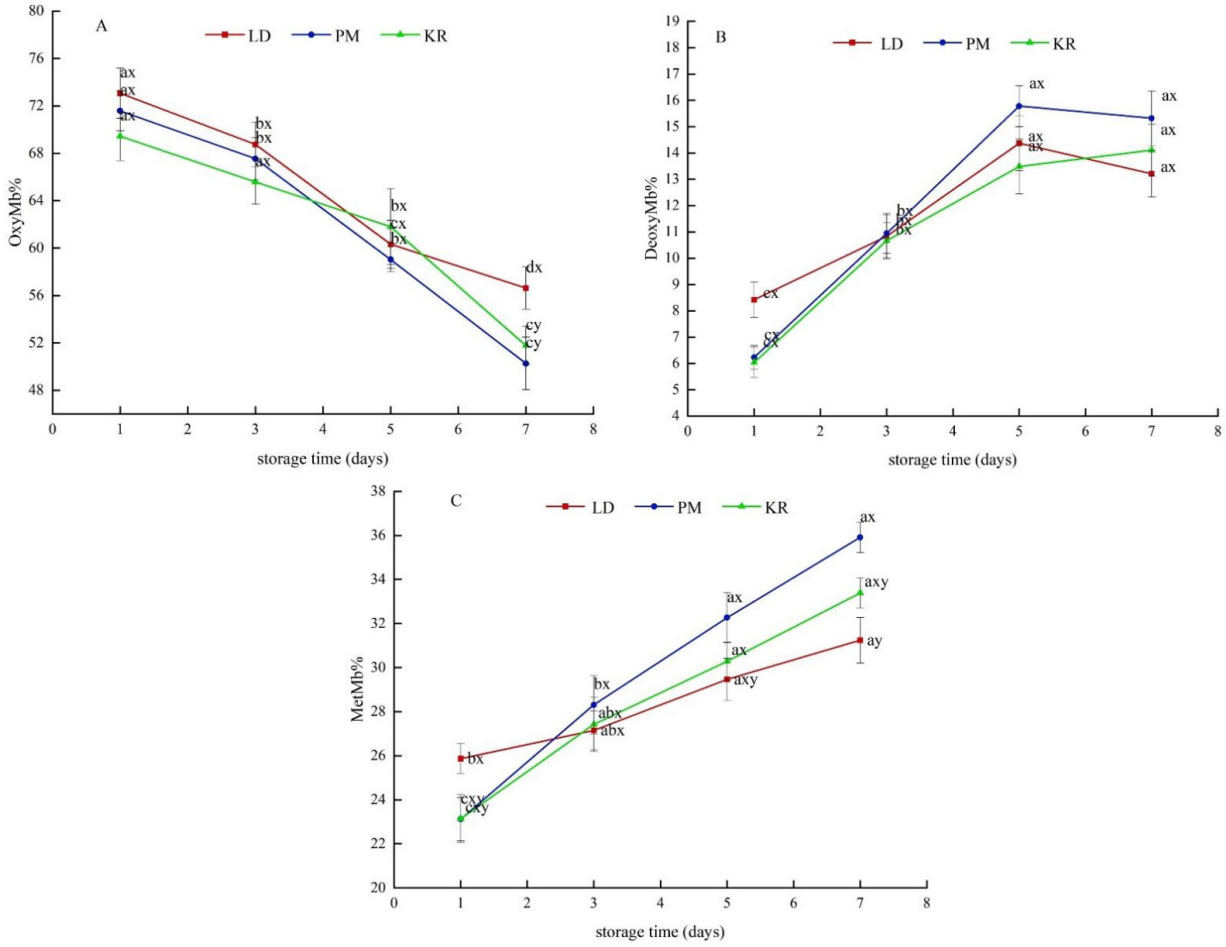

Figure 2. Changes of (A) OxyMb, (B) DeoxyMb and (C) MetMb relative content in different parts of cattle during storage.

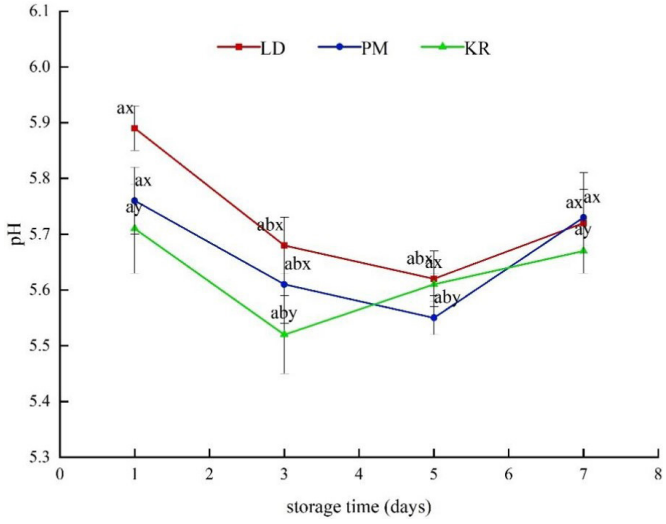

Figure 3. pH changes of different parts of cattle during storage.

3.4 Changes in OCR of cattle from different parts during storage

OCR means that there is mitochondrial respiration in the slaughtered body, and mitochondria will compete with Mb for oxygen, minimizing the amount of oxygen needed to form OxyMb (Gao et al., 2014). It can be seen from Figure 4 that OCR decreases gradually with the prolongation of storage time, which may be due to the decrease of mitochondrial function with the increase of muscle storage time (Li et al., 2020). The LD muscle has better flesh color stability, a^* value (Figure 1B) and lower OCR. It shows that there is a certain relationship between OCR value and meat color stability. The lower the OCR value is, the more a^* value and color stability of muscle can be increased. Some studies have shown that high OCR will lead to the decrease of color stability, because the high oxygen consumption of the mitochondria will reduce the oxygen binding to Mb, thus forming the ideal condition for the formation of MetMb (Holdstock et al., 2023). MetMb accumulates rapidly in muscle, resulting in browning of meat color and decrease of meat color stability. The OCR of the PM muscle in 1~7 d was higher than that of the muscles in the other two parts, and the OCR at 7 d was significantly higher than that ($P < 0.05$) of the LD and KR muscles, so the flesh color stability of the PM muscle was poor, which was consistent with the results of King et al. (2011) found that muscles with higher color stability had lower OCR.

3.5 Changes in cattle total iron, HI and NHI contents in different parts during storage

It can be seen from Figure 5 that the contents of HI (Figure 5A), total iron (Figure 5B) and NHI (Figure 5C) in

different parts of cattle are significantly different ($P < 0.05$). Some studies have shown that the content of HI in different kinds of meat varies, and the content of HI in different parts of the muscle of the same animal is also different (Pereira & Vicente, 2013; Tofteskov et al., 2017). After storage for 7 d, the content of HI decreased and that of NHI increased, which may be due to the gradual transformation of HI into NHI over time. With the extension of storage time, Mb is oxidized, the structure of globin is opened, the connection between heme and globin is weakened (Wu et al., 2017). Mb continues to be oxidized to form NHI (Tian et al., 2022). The contents of total iron and NHI in KR muscle were the lowest ($P < 0.05$). The content of total iron in PM muscle was the highest ($P < 0.05$), and the contents of total iron and HI in LD muscle were lower than those in PM muscle. HI is lower in LD muscle and higher in PM muscle, and the meat color stability of the LD muscle is higher than that of the PM muscle. HI as a catalyst for lipid oxidation accelerates lipid oxidation. An article showed that there was a close relationship between lipid oxidation ability and meat color stability (Benhissi et al., 2020). The higher the degree of lipid oxidation, the lower the stability of meat color, and vice versa (Bu et al., 2022). This shows that the higher HI in PM muscle reduces its meat color stability by accelerating lipid oxidation. Menon et al. (2022) have shown that both HI

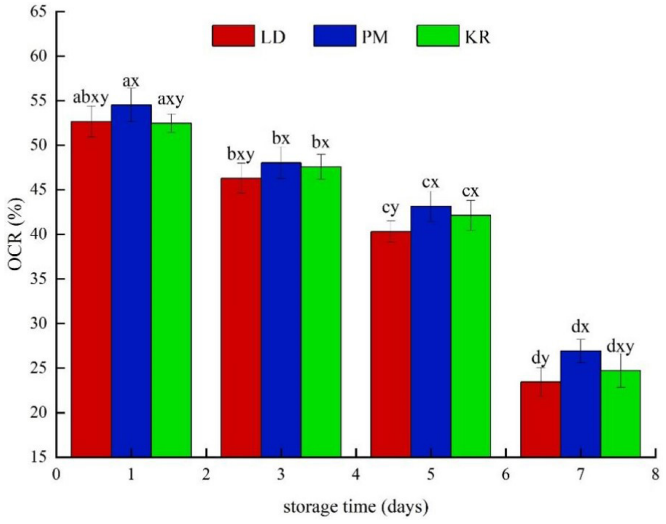

Figure 4. Changes of OCR in different parts of cattle during storage.

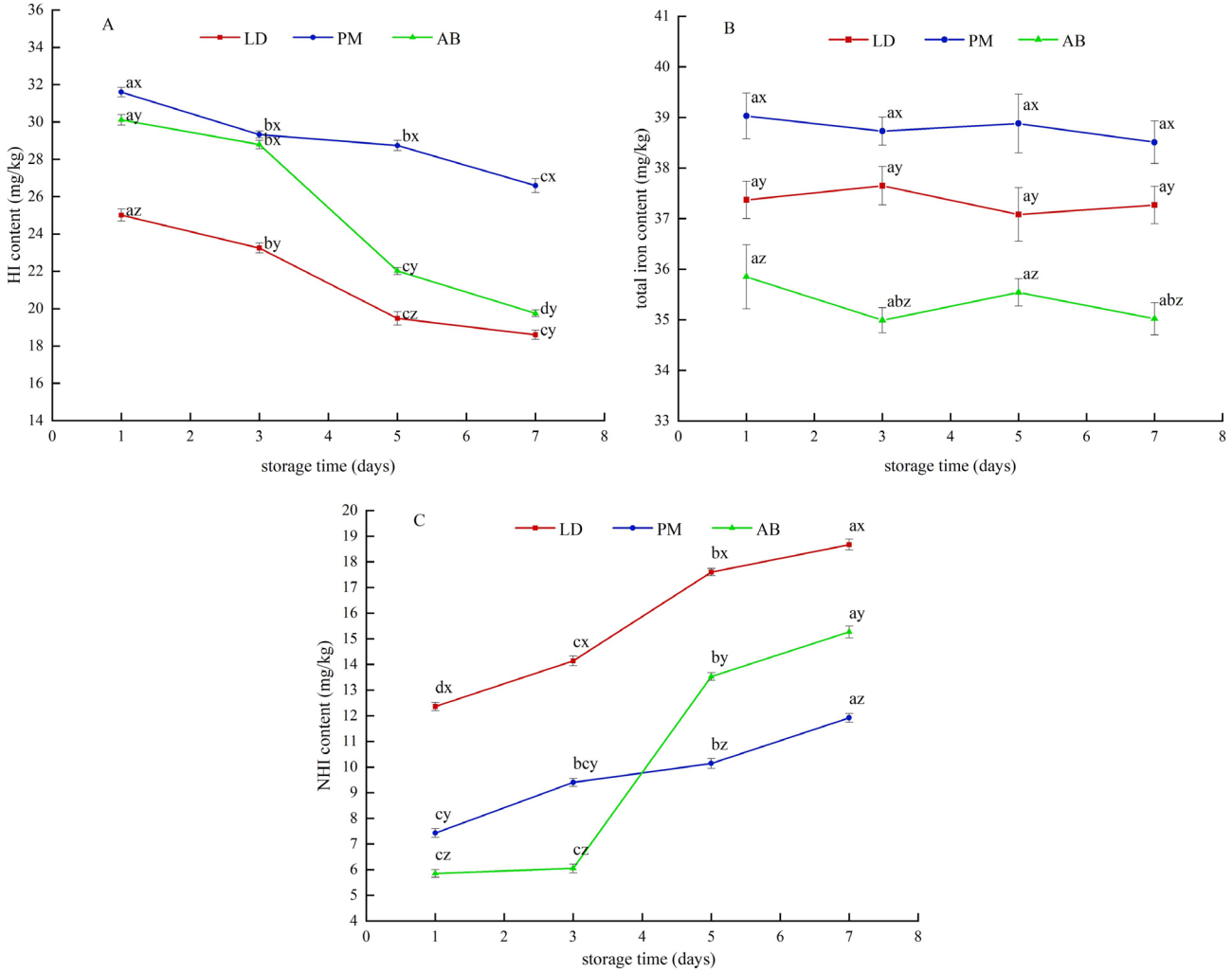

Figure 5. Changes of relative content of (A) HI, (B) total iron and (C) NHI in different parts of cattle during storage.

and NHI accelerate lipid oxidation. Among them, the total iron content of PM muscle is significantly higher than that of the other two parts, so the meat color stability of PM muscle is the worst, which is consistent with the results of OCR, meat color and other indexes. Iron is one of the substances that have great influence on the oxidation rate. The higher content of iron in muscle is related to the higher degree of oxidation of muscle. Iron in muscle affects the stability of meat color by affecting the oxidation of meat. Therefore, the total iron content in muscle can be used as an index of meat oxidation and color stability.

4 Conclusion

Because the muscles of different parts have different physiological functions in vivo, they show different flesh color stability after slaughter. The meat color stability of the three parts from high to low is: LD, KR, PM muscles. The muscles with high chroma, high a^* value and low hue value showed better meat color stability. With the extension of storage time, the relative content of OxyMb in muscle decreases and the relative content of MetMb gradually increases, resulting in a decrease in a^* value and an increase in hue value, which makes the muscle gradually brown. Muscles with low hue values and MetMb relative content show better meat color stability. The high pH value will minimize OxyMb oxidation and maintain better meat color stability, while lower pH value will accelerate the oxidation of Mb and lead to meat color browning. There is a certain relationship between OCR value and meat color stability. The lower the OCR value, the more it increases the a^* value of the muscles and improves the color stability. Higher OCR is beneficial to the formation of MetMb, resulting in the browning of meat color and the decrease of meat color stability. HI and NHI reduce meat color stability by accelerating lipid oxidation. The effect of the change of the meat color index on the color stability of meat was consistent with the effect of iron content on the color stability of meat, therefore the total iron content in muscle can also be used as an index of meat color stability.

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